DEVELOPMENT OF SELF-DECONTAMINATING TEXTILES WITH MICROPOROUS MEMBRANES

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ABSTRACT

Recently a number of compounds have been synthesized that can catalytically break down chemical warfare agents including G-type agents, VX and mustard. These compounds, including enzymes and polyoxometalates display significant level of hydrolytic and or oxidative activity against a wide spectrum of chemical warfare agents. Multispectral protection could be achieved if appropriate catalysts were combined into a clothing system. However, these compounds have been difficult to incorporate into textiles for use in self-decontaminating chemical protective clothing, as their reactivity is severely impacted by the method of attachment onto and into fabrics. We now report a breakthrough in the technology of fiber spinning that has enabled us for the first time to incorporate these catalysts directly into microporous membranes. The new microporous membranes have been developed at the U.S. Army Natick Soldier Center using the process of electrospinning. By electrostatically producing nanofibers from polymer/solvent spinning solutions at room temperature through the application of a high voltage electric field, we have demonstrated increased activity of the original catalyst alone in solution by incorporating the same catalysts into the nanofibers of these new membranes. Activities of enzymes, derivatized enzymes and inorganic catalysts are discussed. Durability of the catalyst with respect to daily use conditions is considered. Manufacturability of these new reactive membranes will be forecast.

INTRODUCTION

Electrospinning is a process for making extremely fine submicron fiber by a process of charging polymer solutions to thousands of volts. This method of manufacturing man-made fibers has been known since 1934, when the first patent on electrospinning was filed by Formhals.[1] Since that time, many patents and publications have been reported on electrospinning.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
00 JAN 2002	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Development Of Self-Decontaminating Textiles With Microporous Membranes	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Natick Soldier Center, Natick, MA 01760-5020 USA; U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD 21010**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
This article is from ADA409494 Proceedings of the 2001 ECBC Scientific Conference on Chemical and Biological Defense Research, 6-8 March , Marriott's Hunt Valley Inn, Hunt Valley, MD., The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**10**	

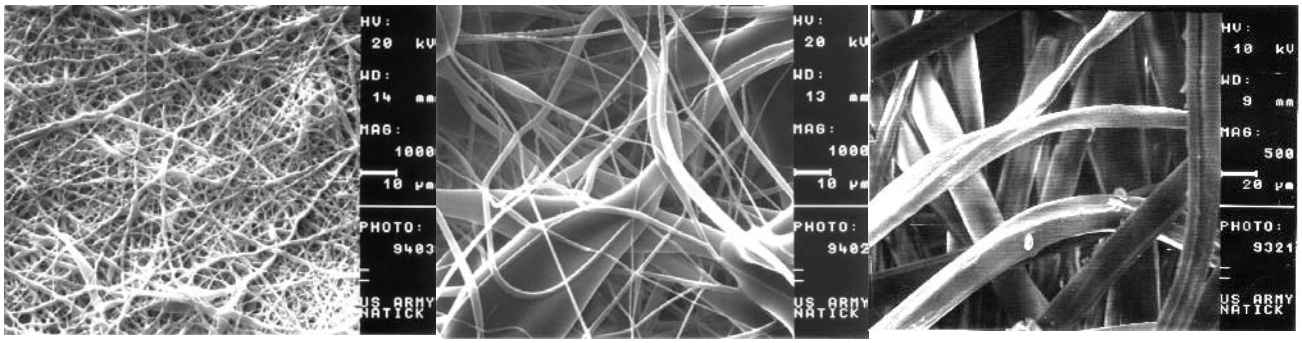
Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

Electrospinning occurs when a polymer solution or melt is charged to high voltage to produce fibers. Voltages of 5kV to 30kV are sufficient to overcome surface tension forces of the polymer, and a free surface of charged polymer will produce fine jets of liquid that are rapidly drawn toward a grounded target. The jet splits a few times near the liquid surface, but before it reaches the target, substantial drawing is observed in a series of looping actions of the rapidly solidifying fiber.[2] The fiber is collected as an interconnected web of small filaments on the surface of a grounded target. The technique has been used for over a decade to produce ultra high efficiency filtration webs.[3-5] It is important to recognize that electrospinning can be used in many other products as well. For example, electrospinning provides the capacity to lace together a variety of types of polymers and fibers to produce ultrathin layers which are useful for protective clothing.[6] Depending on the specific polymer being used, a range of fabric properties, such as strength, weight and porosity, can be achieved. Fiber sizes of 10 nm and smaller have been reported, although lab scale apparatus normally produces fibers from 100nm to 500 to 1.0 μm in diameter. Commercial production size equipment produces fibers in the 0.5 to 10 μm diameter range. Fiber size depends upon solution viscosity, field strength, and field uniformity.[7]

SAMPLE PREPARATION

Various electrospun membranes have been prepared by charge induction of polymer solutions. A positively charged electrode is submerged in a pipette filled with a solution of polymer. In this configuration charged fibers are easily collected over a period of 1-2 hours from a single pipette onto a grounded screen.

The microstructure of an electrospun coating is shown in the scanning electron micrographs (SEM) in Figure 1, exhibiting a range of fiber size and porosities produced by different electrospun polymers. Pellethane fiber sizes range from 0.1-1.0μm in diameter. Estane fibers appear to be 10 times larger, due to the 10% additional enzyme in the material, which substantially thickens the spinning solution and produces larger fiber. Fiber production rates by inducted charge are on the order of 1g fiber per nozzle per hour, depending upon the polymer/solvent combination used. For comparison, cotton fibers are shown in Figure 1c with average fiber diameters of 20μm.



(a) (b) (c)

Figure 1. SEM micrographs at 100x of a) electrospun Pellethane; b) electrospun Estane containing 10% OPAA-C18; and c) cotton fibers at half the magnification of a & b.

Solutions for electrospinning were prepared by dissolving 10 wt% polyurethane in tetrahydrofuran (THF). Two types of polyurethanes were investigated: Pellethane 2103-70A from Dow Plastics, a polytetramethylene glycol ether based thermoplastic elastomer (TPE). Another polyether-based TPE was obtained from B.F. Goodrich Performance Materials, named Estane 58237-80A. Both polyurethanes produce tough, elastic fibers upon electrospinning and readily absorb moisture.

Enzyme was added to this solution immediately prior to electrospinning. Two types of enzymes were used: unmodified organophosphorus acid anhydrolase, OPAA, prepared as previously reported;[8] and OPAA-C18, a modified OPAA encapsulated with a hyperbranched polymer of polyethylene oxazoline with branches of 18-carbon chain lengths.

An inorganic catalyst of a polyoxometalate (POM) compound[9] was incorporated into polymer solutions of TPEs by dissolving 10 wt% POM and varying percentages of the co-oxidant benzoyl peroxide into the solution immediately prior to spinning. The chemical formula for the POM was $H_5PV_2Mo_{10}O_{40}$. Vanadium-based polyoxometalate compounds have been reported to catalytically oxidize thioethers directly to the sulfoxide, and have been under evaluation for the detoxification HD.

All catalyst-loaded TPE solutions were electrospun using 15kV at a target distance of 10 cm to produce a membrane of submicron fibers randomly aligned upon the surface of 5 cm circular aluminum targets. The final fiber mat was easily removed from the aluminum substrate and tested for catalytic activity against appropriate surrogates. OPAA and OPAA-C18 were tested for hydrolytic activity against diisopropyl fluorophosphonate (DFP), a G-agent surrogate. POM catalyst systems were tested for oxidation of 2-chloroethylethylsulfide (CEES), a mustard simulant.

A derivatized OPAA enzyme was used in an experiment to determine the efficiency of binding enzyme directly onto the surface of cellulosic fibers. An electrospun cellulose acetate fiber mat was used and compared to the enzyme binding efficiency of a cotton-based nonwoven fiber mat. Two nonwoven samples prepared by the University of Tennessee Textile and Nonwoven Development Center were used: each were spunbonded (SP) polypropylene (PP) webs with surface bonded cotton fibrils. A web containing 36% cotton was designated as SBPPC36, while a 30% cotton web was

SBPPC30. Cotton fiber sizes from SPPC30 are seen in Figure 1c. Cellulose acetate microfibers were prepared by electrospinning a solution of acetone and 10wt% cellulose acetate (Eastman Chemical sample CA380-30 with a degree of acetyl substitution of 2.5).

Each cotton nonwoven and the electrospun cellulose acetate samples were massed to 10 mg and exposed to a solution of suspended enzymes (protein concentration 71 μg/mL) of OPAA (non-binding) and CBD-OPAA. Cellulose binding domains (CBD) were added to the OPAA enzyme by use of a method of subcloning and preparation of the CBD-OPAA fusion protein from a Novagen (Madison, WI) CBD expression vector.[10] CBD-OPAA can be chemically bound to cotton, while OPAA cannot. After a one hour exposure of the celluosic fibers to the enzyme solutions at room temperature, the nonwovens and electrospun CA were removed, rinsed twice with buffer, and immersed in a solution of acetonitrile with p-nitrophenyl ethylphenyl phosphate (PNEPP), a colorimetric substrate that reacts in the presence of active enzyme.[11]

TESTING METHODS

CBD-OPAA enzyme activity against PNEPP was monitored by UV-VIS spectrometry at 412 nm over a period of 10 minutes. Reaction of the PNEPP substrate with enzyme on the fibers produces a light absorbing product with a molar extinction coefficient of 1300. Product concentration can be related to enzyme activity (rate constant) in mmoles of PNEPP hydrolyzed per gram of material per minute. We report this quantity as Units/g fiber.

In another (non-colorimetric) solution assay, hydrolysis of DFP by OPAA enzyme was measured by a fluoride sensitive electrode (Orion Model EA 940) by placing 1-10mg enzyme-containing material in a 10 mL beaker containing 3.5 mL 0.025M Pipes Buffer (pH 7) and 1.5 mL 0.01 M DFP in deionized water after first recording baseline spontaneous hydrolysis of DFP in water. Concentration of fluoride ion was recorded over a 10 minute period, and the slope of fluoride ion production with time was converted to μmoles of DFP hydrolyzed per minute per mg OPAA contained within the test material. This rate is reported herein as Units/mgOPAA for convenience.

Catalytic oxidation of CEES by POM/benzoyl peroxide (BP) blended into Pellethane fibers and film was determined by the following method. A solution 0.16M CEES was used for oxidation by the POM/BP catalyst systems. During reaction over a period of 24 hours, 1μL aliquots were injected for GC/MS determination of residual CEES concentrations.

RESULTS

1. ACTIVITY OF OPAA ENZYME ENCAPSULATED IN MICROFIBERS

Hydrolysis rates of DFP by OPAA were found to be lower than previously reported[10] due to pH differences. At neutral pH, neat, unmodified OPAA hydrolyzed DFP at an average rate of 7.87 +/- 3.9 Units/mgOPAA. A variation of up to 53% was found in the measurement of activity from run to run. Differences in reaction rates of

neat and modified enzymes are summarized in Table 1 for neat OPAA and OPAA-C18 enzymes in solution, and for enzymes that are encapsulated in electrospun fibers of Pellethane and Estane, or encapsulated in Pellethane film. The apparent activity of neat OPAA encapsulated in fiber is lowered by a factor of 36. When electrospinning neat enzyme in polymer solutions, enzyme does not disperse well and the resulting solution spins poorly, resulting in poor homogeneity in the fibrous membrane. Fiber spinning improves when the modified enzyme, OPAA-C18, is used. The apparent activity of OPAA-C18 in electrospun Pellethane increases 2-fold over the solution activity of the unspun OPAA-C18. When OPAA-C18 is incorporated into Estane and electrospun, the apparent activity increases 3-fold over the solution value for OPAA-C18. There is also a decrease in apparent enzyme activity for a film coating of OPAA-C18 in Pellethane. This indicates that the enzyme is more available for reactions in the electrospun fiber than in a cast film of the same material.

TABLE 1. Effect of Enzyme Encapsulation Upon Hydrolytic Activity.

Sample Treatment	Catalytic Activity (Units/mgOPAA)
Neat OPAA	7.87
OPAA-C18	6.70
Neat OPAA in Pellethane Nanofiber	0.212
Neat OPAA in Estane Nanofiber	1.77
OPAA-C18 in Pellethane Nanofiber	15.76
OPAA-C18 in Estane Nanofiber	18.92
OPAA-C18 in Pellethane Film	4.19

Electrospun enzyme ages and loses activity when encapsulated in polyurethane fiber. Figure 2 shows the effect of room temperature (30^{o}C) aging upon OPAA-C18 apparent activity over a period of one month. Five weeks of aging causes a 4-fold drop in activity of OPAA-C18, falling to 40% of the activity of the original, unspun OPAA-C18.

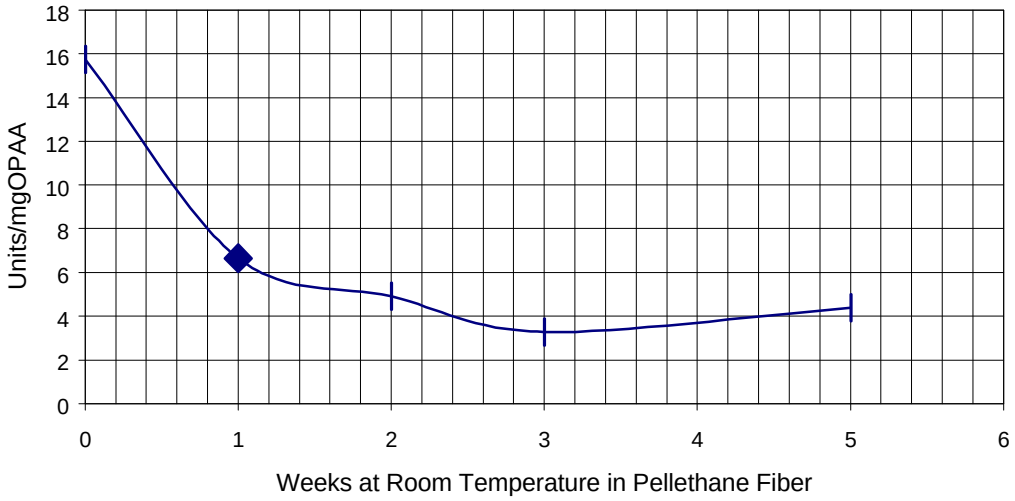


Figure 2. Effect of aging upon enzyme activity for 2% OPAA-C18 in Electrospun Pellethane.

In an effort to determine the fate of the enzyme in the electrospun fibers during hydrolytic reaction against DFP in buffered water, a re-immersion test was performed. A fiber mat of Estane containing 2.5% OPAA-C18 was immersed into a beaker containing buffered water and DFP as described above. After a 10 minute reaction, the fiber mat was removed and residual fluoride ion production in the absence of the enzyme fiber mat was measured. This residual activity was considered to be enzyme that had leached from the fiber mat into solution. The fiber mat from the first test was reimmersed in a new beaker with fresh buffered DFP, and fluoride ion production was measured a second time on the used sample. This reimmersion was repeated 3 times. Figure 3 shows the effect of multiple immersions of the enzyme-loaded fiber mat into the DFP reaction solution. Four immersions, or buffer washings, resulted in a 3-fold drop in apparent enzyme activity. While 16% of the enzyme activity leached into solution after the first immersion, very little leaching was seen after that.

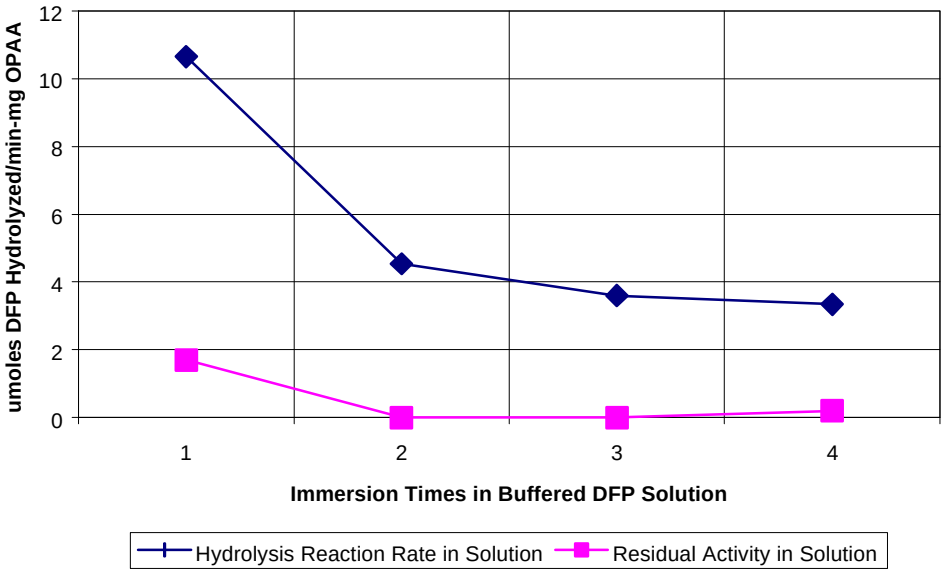


Figure 3. Effect of reimmersion on OPAA-C18 Estane fiber mat activity.

2. ACTIVITY OF CBD-OPAA ENZYME BOUND TO CELLULOSIC FIBERS

For surface bound CBD-OPAA, absorbances of reacted and unreacted PNEPP were monitored to determine a baseline effect of unreacted material. Shown in Figure 4, fibers treated with non-binding OPAA have the same flat slope as the blank solution of PNEPP with no enzyme treated fiber. However, the CBD-OPAA treated fibers react with the PNEPP and an increasing absorbance of PNEPP product with time is recorded.

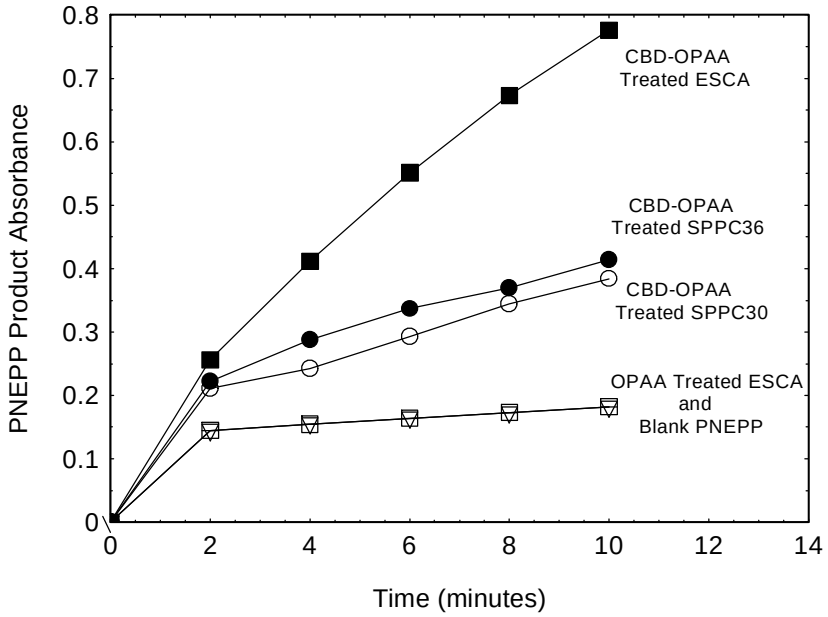


Figure 4. Effect of binding (CBD-OPAA) and non-binding (OPAA) enzyme on breakdown of PNEPP by enzyme-treated electrospun cellulose acetate (ESCA), SPPC36 (36% cotton) and SPPC30 (30% cotton) nonwoven fiber mats.

Table 2 shows the results of residual enzyme activity on the surface of the cotton samples after washing. There is appreciable activity, measured in Units/min-gram of fiber for the CBD-OPAA bound enzyme samples after exposure to PNEPP. Furthermore, there is an apparent increase in enzyme activity for the CBD-OPAA bound to the electrospun cellulose acetate (ESCA), compared to the cotton-surfaced nonwovens. However, further analysis of these results is needed to account for the actual percentage of reactive sites for the CBD-OPAA binding reaction in all three samples.

TABLE 2. Activity of Enzyme-Treated Cellulosic Fabrics Against PNEPP.

Sample	Activity CBD-OPAA (Units/g fiber)
ESCA	39.8
SPPC36	17.7
SPPC30	13.8

3. ACTIVITY OF POLYOXOMETALATE CATALYST IN ELECTROSPUN FIBERS AND CAST FILMS

Oxidation of CEES by POM-containing fibers and films was assessed. Data in Figure 5 show the depletion rate of CEES in solution in the presence of POM in three forms: 1) POM/BP alone in solution, 2) POM/BP in Pellethane cast films, and 3) POM/BP in electrospun Pellethane. We see that a POM to benzoyl peroxide weight ratio of 2:1 works well for the catalyst system alone in the CEES solution. For fibers and films containing the catalyst system, more BP (benzoyl peroxide) is required, possibly due to loss of the peroxide from the fiber and film to the solution. Electrospun fibers containing optimum ratios of POM to benzoyl peroxide will perform better in the catalytic oxidation of CEES in solution. Benzoyl peroxide alone in the nanofiber does not oxidize CEES.

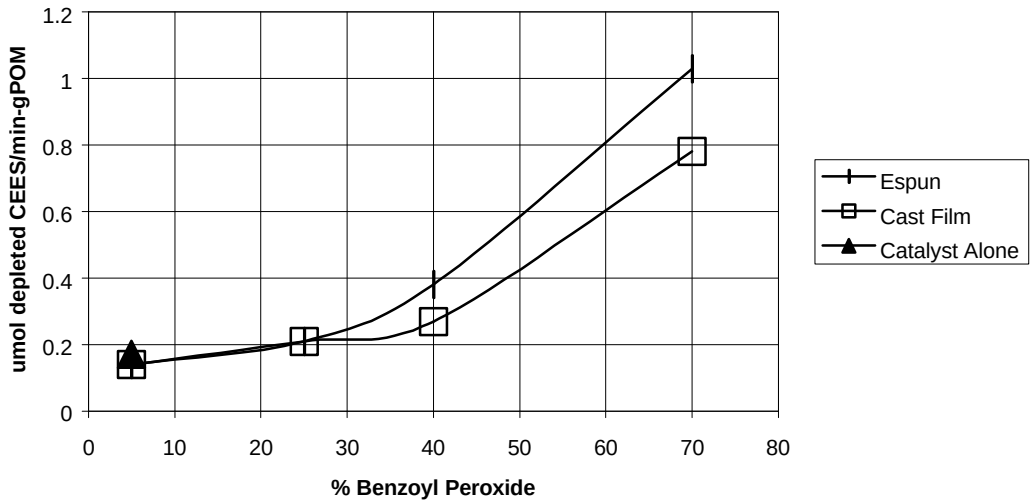


Figure 5. Depletion of CEES by POM/BP catalyst system over a 24-hour period.

CONCLUSIONS

Enzymes and polyoxometalate catalysts have been incorporated into films and nanofibers of polyurethanes. Increased catalytic activity against G-agent and HD surrogates have been found for catalysts encapsulated in nanofibers. Enzymes that have been encapsulated with a hyperbranched polymer are 15-20x more active within the nanofiber than unmodified enzymes. This activity is reduced by 40% after one month of aging. The encapsulated enzymes are not permanently bound in the fiber;16% of the enzyme activity leaches into an initial wash solution, but very little is lost after that.

Cellulose-binding OPAA enzyme has been found to bind effectively to both cellulose acetate and cotton fibers. Electrospun cellulose acetate is capable of binding a high level of enzyme due to the high surface area contained in the microfiber membrane.

Polyoxometalate-peroxide catalyst systems that were encapsulated in polyurethane nanofibers were also more active in the nanofibers, once the peroxide level was adjusted.

Electrospinning has been in use for over 50 years. Nevertheless, it is not used as a method to manufacture microporous membranes. Solvents used during the spinning process are a deterrent to this method of membrane production. Melt processing techniques such as fiber melt blowing might be an attractive option to electrospinning, but will produce much thicker fiber sizes, in the range of 10-100um fiber diameter. However, our preliminary results with enzymes in Estane suggest that these fiber diameters are not detrimental to the reaction of encapsulated catalysts with chemical agents in solution. Inorganic catalysts are continuing to be improved and have the potential to be melt processed into new self decontaminating textiles.

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ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Professor Darrell Reneker at the University of Akron for his expertise and generous sharing of knowledge about the physics and the mechanics of electrospinning.